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Effects of Microwave Irradiation on the Dimensional Stability of Complete Denture Bases

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Effects of Microwave Irradiation on the Dimensional Stability of Complete Denture Bases

by

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Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

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ABBREVIATIONS

W	Watts
ACP	American College of Prosthodontics
S	Streptococcus
SP	Species
MRSA	Methicillin resistant staphylococcus aureus
kHz	Kilohertz
SEM	Scanning electron microscopy
MHz	Megahertz
C	Candida
B	Bacillus
P	Pseudomonas
CFU	Colony forming units
mL	Milliliters
g	Grams
°F	Fahrenheit
°C	Celsius
No	Number
cc	Cubic centimeter
mm	Millimeter
lbs	Pounds
psi	Pounds per square inch

ABSTRACT OF THE THESIS

Effects of Microwave Irradiation on the Dimensional Stability of Complete Denture Bases by

Miguel Pappaterra

Doctor of Dental Surgery, Graduate Program in Prosthodontics
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Dr. Mathew T. Kattadiyil, Chairperson

Introduction: Maintenance for complete dentures is a role that must be undertaken by the patient. It has been determined that two minutes of microwave irradiation at 650 Watts provides denture disinfection, and three minutes provide sterilization[32,34]. There is insufficient data on how this practice affects the long term dimensional stability of commonly used complete denture bases *in the United States*. **Purpose:** The purpose of this study was to perform an *in-vitro* investigation, testing and comparing the effects of microwave irradiation on the dimensional stability of three types of complete denture bases. **Materials and Methods:** An edentulous master cast with reference points was fabricated and duplicated. Thirty maxillary complete dentures were fabricated using three commonly used complete dentures base brands. Dentures were subjected to daily microwave irradiation for three minutes immersed in sterile water. Measurements were made after the specimens were fabricated, and then at one, two, and three months. The dimensional stability of the different complete denture bases were evaluated by comparing the baseline dimensions to the dimensions of each test group at the different time periods. **Statistical Analysis:** The overall dimensional changes of the simulated complete dentures being tested were estimated by using the percentage difference between the baseline area of each denture base and each test group. Data was statistically analyzed using a 2-way repeated measures ANOVA; followed by the Tukey HSD test. Statistical analysis was conducted at the 95% level of confidence

(α=.05). **Results:** When comparing to the baseline, the Eclipse group had a mean shrinkage of 0.083% at one month with a standard error of 0.021; 0.14% (0.025) of shrinkage at two months; and 0.23% (0.026) of shrinkage at three months. The Ivocap group had 0.069% (0.013) shrinkage at one month; 0.16% (0.015) shrinkage at two months; and 0.25% (0.025) of shrinkage at three months. And the Lucitone group had 0.13% (0.017) of shrinkage at one month; 0.19% (0.023) of shrinkage at two months; and 0.33% (0.023) of shrinkage at three months. **Conclusion:** Within the limitations of this study, we can conclude that microwave irradiation did not reveal clinical significance on the dimensional stability of Eclipse, Ivocap and Lucitone denture bases when used at the wattage & time settings used in this study. Eclipse demonstrated the least amount of distortion among the denture bases tested.

CHAPTER ONE

OBJECTIVE OF THE STUDY

This study investigated the effects of microwave irradiation on the dimensional stability of different complete denture bases.

Null Hypothesis and Specific Aims

Null Hypothesis:

1. Microwave irradiation will not affect the dimensional stability of complete denture bases.

Specific Aims:

1. To compare the effects microwave irradiation on the dimensional stability of three different complete denture base materials.
2. To determine which complete denture base will have the least amount of distortion when subjected to microwave disinfection.
3. To characterize the effects of microwave irradiation on complete denture bases at three months.

Statement of the Problem

For centuries removable prostheses have been an accepted treatment for replacing missing teeth on fully or partially edentulous patients.

Although patients may be fully edentulous, there is a tendency for the oral flora of natural dentitions to remain in edentulous or partially edentulous patients present. There are reports in the prosthodontic literature that this flora is often associated with an increased incidence of denture stomatitis[2-4] and other serious diseases such as bacterial endocarditis, aspiration pneumonia, chronic obstructive pulmonary disease, generalized infections of the respiratory tract, rheumatoid arthritis and other systemic diseases[5,6]. There is also a correlation between edentulism and a risk of head and neck, lung, and esophageal cancer[7]. Failure to properly clean the accumulated denture biofilm subjects the patient to a risk of contracting any of these serious diseases.

In an effort to prevent disease, several ways of cleaning dentures have been proposed[1]. Brushing with denture creams and pastes, soaking and brushing with commercially available denture cleansers (effervescent tablets), ultrasonic cleaning and microwave irradiation are varied methods of cleaning dentures. However, studies have determined that these mechanical cleaning methods only reduces the microbiological biomass on the denture surface[9-16]. Ultrasonic cleaning and microwave irradiation are the most appropriate ways to properly disinfect complete dentures[17]. Cleaning the denture with ultrasonic units and solutions are available in dental offices and dental laboratories. In contrast, at-home microwave irradiation of the appliance may be successfully employed for regular and complete disinfection of a person's complete denture.

A publication by the American College of Prosthodontists[1] (2011) expresses a need for further research and exploration of microwave cleaning for the purpose of improving the quality and safety of denture use. Unfortunately very few publications investigating this topic exist in the dental literature. None of these publications have

evaluated commonly used complete denture bases. Furthermore, no studies have been published on the long term effect of microwave irradiation in complete dentures.

This research provides values of any dimensional damages of three complete denture base brands when they are subjected to long-term microwave irradiation. The results of the research gives guidelines to clinicians to educate denture wearers on the proper methods of care and maintenance of their prostheses.

CHAPTER TWO

REVIEW OF LITERATURE

In 2009, the American College of Prosthodontists (ACP) formed a task force to develop contemporary, evidence-based guidelines for the care and maintenance of complete dentures. This task force comprised individuals representing the ACP, the Council on Scientific Affairs of the American Dental Association, the Academy of General Dentistry, the American Dental Hygienists' Association, the National Association of Dental Laboratories, and representatives from GlaxoSmithKline Consumer Healthcare. A comprehensive literature search was conducted by the task force members using PubMed, EMBASE, known prosthodontic references and materials obtained from the U.S. Centers for Disease Control and Prevention[1].

Among other critical subjects related to complete dentures Felton et al[1] discussed, denture biofilms and denture cleaning methods.

Dentures accumulate plaque, stain and calculus similar to the natural dentition. Failure to properly clean the accumulated denture accretions, adherent material and calcified plaque aggregation of microorganisms, is associated with an increased prevalence of localized denture stomatitis[2-4]. In addition, these accretions may render a patient susceptible to serious systemic diseases, including bacterial endocarditis, aspiration pneumonia, chronic obstructive pulmonary disease, generalized infections of the respiratory tract and rheumatoid arthritis[5,6]. There appears to be a strong correlation between edentulism (and denture biofilm) and risk of head and neck, lung, and esophageal cancer[7].

Denture plaque is a complex aggregate of oral bacteria, fungi and other organisms. It is estimated to contain more than 1,011 organisms per milligram (wet weight) involving more than 30 different species. While there is general consensus that the composition of denture plaque is similar to that of oral plaque in the dentate patient, the biomass composition may vary among individuals, and between sites in the oral cavity and sites on the dentures in the same individual. It has been determined that oral biofilms accumulate more readily on rough denture surfaces than on smooth ones.

In a 2009 *in vitro* study by Charman and et al[8], denture acrylic resin samples were prepared to four different degrees of surface roughness, and the *Streptococcus oralis* was cultured on these surfaces. The study demonstrated that there was increased coverage of the denture with *S. oralis* bacteria as the surface roughness increased, and that heat-processed denture base acrylic was less likely to allow growth of organisms than were cold-cured resin bases. The study demonstrates a significant effect of surface roughness on the efficacy of denture cleaning, overall denture hygiene effectiveness and rate of biofilm reformation of varied cleaning regimens. The results also indicate that non-abrasive cleansers may offer a more appropriate cleaning regimen. These authors concluded that: (1) care should be taken not to scratch the surface of processed denture bases or acrylic prosthetic denture teeth during processing or use. (2) One must realize that the intaglio surface of the denture base, surface contacting the oral tissues is never polished completely smooth.

Felton et al[1] in his review article also identified four ways of cleaning dentures: (1) brushing with denture creams and pastes, (2) soaking and brushing with commercially available denture cleansers (effervescent tablets), (3) ultrasonic cleaning and (4)

alternative denture cleansing methods. The alternative methods encompass microwave irradiation and boiling the denture. Boiling dentures has been shown to deform the base, rendering it unusable.

Because of the defined relationship of biofilm to stomatitis, dentists and healthcare providers must carefully instruct the edentulous patient in the proper methods for cleaning and maintaining dentures.

The characteristics of an ideal denture cleanser should demonstrate activity to remove biofilm and stains. The cleanser should be *antibacterial* and *antifungal* to minimize the level of biofilm and potentially harmful pathogens below clinically relevant levels. The acceptable clinical level of pathogens on the denture surface has yet to be adequately defined. Ideally, the denture cleanser should be: (1) nontoxic, (2) compatible with denture materials, (3) not modifying (roughen or degrade) the surface of the acrylic resin denture base or prosthetic teeth, (4) short acting (≤8 hours), (5) easy to use by the patient or caregiver, (6) an agreeable taste and (7) cost effective.

The efficacy of brushing with denture creams and pastes has well reported. Dills et al[9] (1988) research compared the ability of two most popular contemporary methods for denture cleaning to remove plaque microorganisms from dentures. Dentu-Creme® abrasive denture paste and Efferdent® alkaline peroxide denture-cleanser soak were selected for the study. The authors found that combining brushing with soaking did not reduce significantly the level of recoverable microorganisms more than soaking alone. Similarly, brushing alone did not consistently remove more microorganisms than were observed in a no-treatment group. The denture-cleanser soak displayed broad antimicrobial activity against gram-negative anaerobic rods (*Fusobacterium sp.*), gram-

positive facultative cocci (*Streptococcus sp.)*, and gram-negative anaerobic cocci (*Veillonella sp.*), and total recoverable microorganisms. Cell species were all equally reduced by the denture-cleanser treatment. These results support the advantage of using a denture cleanser combined w/ brushing with a denture paste for proper denture hygiene effectiveness.

In 2004 Barnabé et al[10] studied the efficacy of sodium hypochlorite and coconut soap used as disinfecting brushing agents in the reduction of denture stomatitis. The microorganisms studied were *Streptococcus mutans* and *Candida albicans*. They found that coconut soap and 0.05% sodium hypochlorite significantly reduced clinical signs of denture stomatitis. However, counts of *Candida albicans* were not reduced, *Streptococcus mutans* were reduced but not significantly. Thus, *Candida albicans* appears to be resistant to anti-microbial debridement from the denture base. Other methods of denture cleansing appear superior to this method, and the abrasiveness of these denture pastes is of concern.

In 2009 Panzeri et al[11] studied the physical properties of two experimental dentifrices for complete denture hygiene, their effect on denture biofilm removal and antimicrobial properties in a clinical trial. The dentifrices tested were comprised of two solutions. One was based on the addition of 1% chloramine and the other 0.01% fluorosurfactant. They concluded that brushing complete dentures with the experimental dentifrices tested could be effective for the removal of denture biofilm. However, no treatment influenced *Candida albicans* or *non-albicans species*.

Commercially available denture cleansers include varied active agents to remove biofilm from dentures. Included agents are hypochlorites, peroxides, enzymes, acids and

oral mouth rinses. Each of these immersion cleansers has a different mode of action and a different rate of efficacy for removal of adherent denture biofilms. While commercially available denture cleansers were capable of reducing the biomass present on dentures, none of the *in vivo* studies reviewed demonstrated that any of the methods or products used was bactericidal. *In vitro* studies, however, have demonstrated that sodium hypochlorite (bleach) was superior to all other types of commercially available denture cleansers.

DePaola et al[12] in 1990 evaluated the effects of an antimicrobial mouthrinse, denture soft relines, and a placebo rinse on clinical findings and microbial flora of 78 patients with denture stomatitis. In the absence of other mechanical denture hygiene procedures, the antiseptic rinse and regular change of relines were equally effective in reducing denture stomatitis and potential pathogens of denture plaque flora.

In 1999 Lin et al[13] investigated the efficacy of a commercially prepared microbial disinfectant Alcide® on the external and internal surfaces of acrylic resins. Analysis was performed with microbial colony counts, SEM, and statistical analyses. Viable microorganisms still remained on the internal and external surfaces of treated resins. They concluded that chlorine dioxide reduces, but does not eliminate, viable microorganisms on the dental prostheses tested.

In 2003 Pavarina et al[14] evaluated the effectiveness of immersion solutions alone. Chlorhexidine gluconate, sodium hypochlorite, iodophors and alkaline peroxide were tested. They found that all the mandibular dentures tested immersed in iodophors showed positive growth of the microorganisms; and the maxillary dentures tested were positive for growth in six of eight dentures. The chlorhexidine gluconate, sodium hypochlorite

and alkaline peroxide solutions were proven to be effective in reducing the growth of the microorganisms following ten minutes of immersion periods.

Harrison et al[15] (2004) did an *in vitro* study of the effect of a limited range of denture cleaners on denture surface roughness and removal of Candida albicans from conventional heat-cured acrylic resin denture base material. The denture cleaners evaluated were conventional toothpaste, toothpaste with stain remover, denture cleaning paste and an immersion type cleaner. The immersion type cleanser was found to be the most suitable cleaner of the denture base material because of its low abrasivity and effective removal of organic debris. Paste type cleaners were found to significantly roughen the denture base material. However, they didn't evaluate the ability of the denture cleaners used to inhibit the growth of plaque.

In 2007, Maeda et al[16] examined the survival dynamics of several epidemic health care-associated and community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) in a planktonic state in widely employed denture-cleaning solutions. The study suggested that the formulations tested may be useful in lowering the numbers of MRSA.

Ultrasonic cleaning of dentures occurs frequently in both the dental office and the dental laboratory. The mode of action of ultrasonic devices is unique in that they produce ultrasonic sound waves (20 to 120 kHz), which create microscopic cavities (bubbles) that grow and implode. This implosion creates voids that result in localized areas of suction. Materials adhering to the denture are loosened and removed by this action.

Two representative types of solutions that are commercially available for use in the ultrasonic cleaner are BioSonic Enzymatic® (Coltene/Whaledent™; Cuyahoga Falls, OH), which contains nonionic detergents, protease enzymes and 400 parts per million

isopropyl alcohol, and Ultra-Kleen® (Sterilex™; Hunt Valley, MD), which requires the mixing of two solutions that results in the formation of an alkaline-peroxide cleanser. Ultrasonic denture cleaning has been reported in the literature.

In 2005 Muqbil et al[17] assessed the antimicrobial activity of two cleaning solutions and tap water after varying periods of use in one ultrasonic cleaner. Using an ultrasonic apparatus, they found that killing became less effective on repeated use of a commercial cleaning solution. Reduction was highest when fresh ultrasonic cleaning solutions were used. In no case did complete sterilization occur. In addition to removing adherent material, ultrasonic cleaning may also markedly reduce the number of viable organisms present.

The first article to investigate the application of microwave irradiation in dental offices was by Rohrer and Bulard[18] in 1985. The removal of adherent biofilm, microorganisms on dentures, dental burs and air turbine handpieces was tested. They cultured fungi, viruses, and aerobic and anaerobic bacteria (including spore formers) and then irradiated them using 720 W at different exposure times. They concluded that microwave irradiation was a proper way to obtain sterilization. They also looked at dimensional stability changes of dentures and concluded that there were absolutely no dimensional changes, even after 100 exposures of at least eight minute periods for one particular mandibular denture tested. However, the authors didn't mention what types of acrylic denture bases were used, and the sample size was only one.

Burns et al[19] in 1990 did a study on dimensional stability of acrylic resin materials after microwave sterilization. The objective of the study was to measure and determine the potential influence of microwave sterilization on the dimensional stability of

polymerized acrylic resin denture base materials. Uniform samples of previously heat-polymerized, autopolymerized, and visible-light-polymerized acrylic resins were measured for changes in weight and length before and after microwave exposure. All three acrylic resin materials maintained excellent stability; all materials had shrinkage values in the range of 0.02% to 0.03%. It was concluded that the shrinkage obtained from microwave irradiation is clinically insignificant compared to polymerization shrinkage, which averages 0.2%. However, they only included one brand of the denture bases of the present study, Lucitone199® Dentsply (York, PA) and the samples used were not conventional dentures.

In 1991 Najdovski et al[20] investigated microwave irradiation at 325 W, 650 W and 1400 W power on *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*, and spores of *Bacillus subtilis* and *Bacillis stearothermophilus*. Bacterial spores were only killed in aqueous suspension when a 1400 W setting was used for 10 to 20 minutes. They concluded that conventional microwave ovens available on the market may be used for a high level of disinfection but not for sterilization, and only then if sufficient water is present.

In 1995 Polyzois et al[21] examined the effect of glutaraldehyde and microwave disinfection on the dimensional stability, flexural properties, and microhardness of a heat-polymerized denture acrylic resin. They concluded that all specimens exhibited linear changes during disinfection procedures. Although these changes were statistically significant, they are not of clinical importance (< 0.03%). The flexural properties (strength, modulus, and deflection) remained unaffected during all disinfection procedures. The small microhardness differences observed among the various

disinfecting procedures are considered not to be clinically significant. However, they didn't use simulated dentures and the materials used were different from the present study.

In 1998 Webb et al[22] did an *in vitro* test of the efficacy of microwave irradiation with sodium hypochlorite soak for the removal of adherent biofilm. They used scanning electron microscopy (SEM) to evaluate their results. Microbiological analyses showed that the inoculated dentures became sterile after six minutes of irradiation at medium setting (2450 MHz, 350 W). Those that were soaked for eight hours in either 0.02% or 0.0125% sodium hypochlorite, microbiological analyses showed that the experimental dentures inoculated with *C. albicans* became sterile. By contrast, those inoculated with *S. gordonii* did not become sterile. The results of this study indicate that microwaving may be a more effective method of denture sterilization than denture soaking in sodium hypochlorite. Compared with microwaving, hypochlorite reduces more the levels of residual non-viable microorganisms attached to the denture surface. However, the long term effect of hypochlorite soak on the color stability of the denture was not mentioned.

Dixon et al[23] (1999) investigated the efficacy of microwave irradiation against *C. albicans* colonized on three soft denture liners (Permaflex®, PermaSoft® and Molloplast-B®) and one heat-polymerized denture base resin (Lucitone 199®), and the effect of this irradiation on the hardness of the materials tested. They concluded that five minutes of microwave irradiation, while immersed in water, killed all *C. albicans* present on the materials tested. Repeated five minute irradiation significantly affected the hardness of only one of the materials tested (PermaSoft®).

In 2001 Banting et al[24] compared the use of microwave irradiation versus chlorhexidine digluconate in patients with a positive test for *C. albicans*. The results of this study indicated that microwaving a complete maxillary denture was found to be more effective than soaking it in 0.2% chlorhexidine solution for eradicating the invasive form of the *C. albicans* organism. Re-infestation of the denture surface and infection of the adjacent soft tissue were delayed dramatically in patients whose dentures were microwaved. Subjects in the microwave group were less likely to have *C. albicans pseudohyphae* on their palatal mucosa as well as on the tissue surface of the maxillary denture compared with soak group subjects. They concluded that given that microwaving is less expensive (exclusive of capital costs for equipment), more convenient, and requires considerably less effort on the part of the caregiver, it should be considered as a practical procedure for disinfecting complete dentures as an adjunct to the treatment of oral candidiasis.

In 2003 Goodson et al[25] examined the effectiveness of a denture sanitizer when used in combination with a microwaving procedure. The statistical results indicated that the dentures were decontaminated most effectively when the denture sanitizer was used in conjunction with a two-minute microwave procedure.

In 2005 Pavarina et al[26] investigated the effect of microwave disinfection (650 W/6 min) on the flexural strength of five hard chairside reline resins (Kooliner®, Duraliner II®, Tokuso Rebase Fast®, Ufi Gel Hard®, New Truliner®) and one denture base resin (Lucitone 550®). They found that flexural strength of the material Tokuso Rebase® was not significantly affected by microwave irradiation. Seven cycles of microwave disinfection resulted in a significant decrease in the flexural strength of

material Duraliner II®. Material Ufi Gel Hard® was the only resin detrimentally affected by microwave disinfection after two and seven cycles. However this study predominantly looked at chairside reline resins and one week of microwave irradiation.

In 2005 Pavan et al[27] evaluated the influence of microwave treatment on dimensional accuracy along the posterior palatal border of maxillary acrylic resin denture bases processed by water-bath curing. The existence of gaps between the casts and acrylic bases was assessed using a profile projector at five points. Treatment in a microwave oven at 604 W for ten minutes produced the greatest discrepancies in the adaptation of maxillary acrylic resin denture bases to the stone casts.

In 2005 Campanha et al[28] stated that acrylic resin denture teeth soften upon immersion in water, and the heating generated during microwave sterilization might enhance this process. They examined six different denture teeth and found that specimens immersed in water for 90 days and two cycles of microwave sterilization had no effect on the hardness of most of the acrylic resin denture teeth.

In 2006 Silva et al[29] evaluated the effectiveness of microwave irradiation on the disinfection of simulated complete dentures. Dentures were inoculated with Candida albicans, Streptoccus auerus, Bacillus subtilis and Pseudomonas aeruginosa. They submitted dentures to six minutes of microwave irradiation at 650W. Sterilization was obtained for dentures contamined with *S. aureus* and *C. albicans* and disinfection was obtained for dentures contamined with *B. subtilis* and *P. aeruginosa.*

In 2007 Seo et al[30] evaluated the dimensional stability of intact and relined acrylic resin denture bases after microwave disinfection. Microwave irradiation was performed for six minutes at 650 W for seven days. They found that microwave disinfection

produced increased shrinkage of intact specimens and those relined with New Truliner® and Kooliner®. They concluded that the clinical significance of the results obtained is difficult to interpret, such contractions may cause pressure on the supporting tissues and, thus, discomfort to the patient. Consequently, clinicians should be aware that additional denture base adjustments are likely be required after microwave disinfection. They recommended a long-term controlled clinical study conducted to establish correlation with these laboratory findings and, thus, determine the applicability of microwave irradiation as a method for denture disinfection.

In 2008 Dovigo et al[31] evaluated the effectiveness of microwave irradiation for disinfection of simulated complete dentures. The microorganisms tested in this study were *S. aureus*, *P. aeruginosa* and *B. subtilis*. The simulated dentures were exposed to microwave irradiation at 650 W in exposure times of three and five minutes. They found out that microwave irradiation for three minutes at 650 W produced sterilization of complete dentures contaminated with *S. aureus* and *P. aeruginosa*. Dentures contaminated with *B. subtilis* were disinfected by microwave irradiation after three and five minutes at 650 W.

Mima et al[32] in 2008 evaluated the effectiveness of different exposure times of microwave irradiation on the disinfection of a hard chairside reline resin. Sterile specimens were individually inoculated with one of the tested microorganisms (*P. aeruginosa*, *S. aureus, C. albicans*, and *B. subtilis*). Irradiated specimens were immersed in water and microwaved at 650 W for one, two, three, four, or five minutes before serial dilutions and platings. Irradiated specimens were also incubated for seven days. Some specimens were prepared for scanning electron microscopic (SEM) analysis. As a result,

specimens irradiated for three, four, and five minutes showed sterilization. After two minutes of irradiation, specimens inoculated with *C. albicans* were sterilized, whereas those inoculated with bacteria were disinfected. One minute of irradiation resulted in growth of all microorganisms. SEM examination indicated alteration in cell morphology of sterilized specimens. The effectiveness of microwave irradiation was improved as the exposure time increased. This study suggested that three minutes of microwave irradiation can be used for acrylic resin sterilization, thus preventing cross-contamination. However, they didn't used simulated complete dentures and they only tested one chairside acrylic resin reline material, Tokuso Rebase Fast Set® (Tokuyama Dental Corp., Tokyo, Japan).

Sanitá et al[33] (2008) evaluated the effectiveness of microwave irradiation on the disinfection of simulated complete dentures inoculated with American Type Culture Collection (ATCC) and HIV isolates of five species of *Candida* (*C. albicans, C. dubliniensis, C. krusei, C. glabrata and C. tropicalis*). They concluded that microwave irradiation for three minutes at 650 W resulted in sterilization of all complete dentures.

Ribeiro et al[34] in 2009 evaluated the clinical effectiveness of two exposure times of microwave irradiation on the disinfection of complete dentures. They collected biofilm samples from dentures of 30 patients, who were randomly divided into two experimental groups of 15 subjects each: Group 1-patients had their maxillary denture microwaved for three minutes (650W); Group 2-patients had their maxillary denture microwaved for two minutes (650W). Denture biofilm samples were taken with swabs, before and after microwave irradiation. All microbial material was plated on selective media for Candida spp., *Staphylococcus sp., S. mutans* and a non-selective media. After incubation (48

hours/37 degrees Celsius), the number of colony-forming units (cfu/mL) was counted. Microorganisms which grew on selective media were identified using biochemical methods. As a result they found that microwave irradiation for three minutes resulted in sterilization of all dentures evaluated. After microwave irradiation for two minutes, a significant decrease in *Candida sp.*, *Staphylococcus sp*., *S. mutans* and non-identified species was achieved in comparison with the cfu/mL obtained before irradiation. The colonies grown after two minutes of microwave irradiation were identified as *C. albicans*, *non-aureus Staphylococci* and *S. mutans*. They concluded that microwave irradiation for three minutes may be a potential treatment to prevent cross-contamination. However, they didn't report the type of acrylic resin denture bases studied. They suggested that further studies should be performed to confirm the effects of repeated cycles on the integrity of dentures.

Senna et al[35] in 2010 evaluated the influence of the area of *C. albicans* biofilm on denture disinfection by microwave energy. Specimens were irradiated at a power of 450, 630 or 900 W for different time intervals (one, two or three minutes). Dentures with small areas of biofilm were disinfected after one minute at 900 W and two minutes at 450 or 630 W. A positive correlation was found between water temperature and effectiveness of disinfection. They concluded dentures with larger biofilm areas required longer irradiation exposure to be disinfected.

In 2010 Basso et al[36] evaluated the effect of microwave irradiation on the linear dimensional stability of complete dentures. Microwave irradiation was performed at 650 W for three minutes once a week and three times a week for a month. Changes were reported, but because they were less than 1% there was no clinical significance.

In 2012 Senna et al[37] evaluated if adding an enzymatic cleanser to a microwave disinfection regimen would disinfect dentures with shorter irradiation time. The enzymatic cleanser tested was Polident® 3-min GlaxoSmithKline™ (Philadelphia, PA). They concluded that adding the enzymatic cleanser tested is efficient in disinfecting dentures at lower irradiation time and temperature. However, only one microorganism was tested (*C. albicans*) using one enzymatic cleanser.

Altieri et al[38] in 2012 evaluated the efficacy of two disinfectant solutions and microwave irradiation in disinfecting simulated complete. The authors contaminated 36 simulated complete dentures with MRSA and divided them into four equal groups: (1) a positive control group consisting of dentures that were not disinfected; (2) a group that soaked in one percent sodium hypochlorite for ten minutes; (3) a group that soaked in two percent chlorhexidine gluconate for ten minutes; (4) and a group that underwent microwave irradiation at 650 W for three minutes. As a result, all dentures from the control group (no disinfection) showed substantial microbial growth on the plate. The authors observed no evidence of microbial growth on plates of any disinfected dentures. After seven days of incubation, the authors observed broth turbidity in all beakers containing the dentures disinfected with one percent sodium hypochlorite. They concluded that soaking in chlorhexidine gluconate solution and microwave irradiation resulted in complete disinfection of all dentures contaminated with MRSA in both the short (two days) and the long term (seven days). Soaking in sodium hypochlorite solution was effective only as a short-term disinfectant.

CHAPTER THREE

MATERIALS AND METHODS

Three resin denture base brands that are commonly used in the United States were selected for this study. Each denture base selected has its own processing protocol. The name of the resins, manufacturers, proportions of powder to liquid, composition of monomer and polymer, and the polymerization condition recommended by the manufacturers are listed in Table 1.

Table 1. Denture bases.

Product	Manufacturer	Type	Powder: Liquid Ratio (g:ml)	Polymerization Condition	Composition
Lucitone199®	Dentsply™ (York, PA)	Heat-polymerized acrylic resin	21g /10 ml	1st stage: 90 minutes at 163°F (73°C) 2nd stage: 30 minutes at 212°F (100°C)	Powder: Polymethylmethacrylate Liquid:Methyl Methacrylate, Ethylene Glycol Dimethacrylate
Eclipse®	Dentsply™ (York, PA)	Light-cured resin	N/A	Eclipse Processing Unit® Dentsply™ for 60 minutes	Urethane Methacrylate , Stearyl Acrylate
SR Ivocap®	Ivoclar-Vivadent™(NY, USA)	Heat-polymerized acrylic resin injection system	20g / 30 ml	1st stage: 35 minutes at 212°F (100°C) 2nd stage: 30 minutes at 50°F (10°C)	Powder: Polymethylmethacrylate Liquid: Methyl Methacrylate, Ethylene Dimethacrylate

Simulated complete maxillary dentures were fabricated following the methodology of a previous study by Silva et al[29]. A total of 30 simulated maxillary complete dentures were fabricated, ten of each of the denture bases selected.

A cast stone model was fabricated from a maxillary edentulous rubber mold (model EDE1001; Nissin™, Japan, *figure 1*).Three round depressions were made with an acrylic bur (model H251E; Brasseler™; Savannah, GA) on the land area of the stone cast obtained. These indentations served as indexes in the fabrication of the maxillary wax-trial complete dentures. Five markers were placed on different locations of the alveolar ridge. These markers were round in form. These markers were prepared using a No.4 carbide round bur (Brasseler™; Savannah, GA). After denture processing, these markers were located on the intaglio surface of the processed dentures and were used for measurements (*figure 2)*.

Figure 1. Nissin™ Edentulous Jaw.

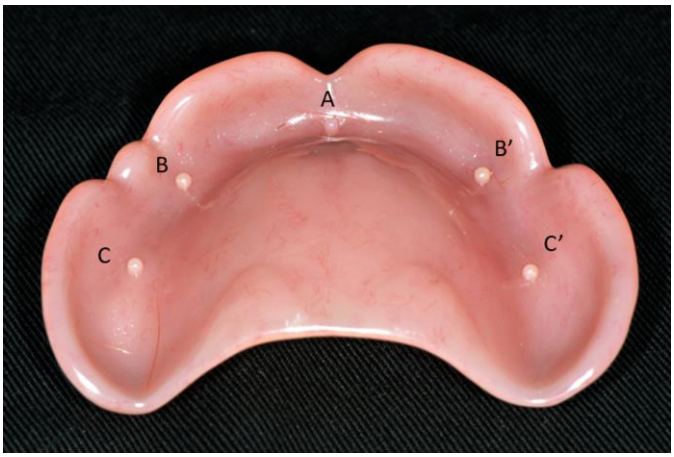


Figure 2. Markers in place after fabrication of a sample.

A new mold was made of the modified edentulous cast using a polyvinyl siloxane material used to duplicate casts (Capsil™ Quick Set A & B Kit; Great Lake Orthodontics; Tonawanda, NY). 30 stone casts were poured using type III dental stone (Microstone Gold® Whip Mix Corporation™; Louisville, KY). On one of the master casts, a simulated maxillary complete denture base and occlusal rim was waxed (*figure 3)*. This waxed-trial denture was duplicated using a high-viscosity VPS (Splash!® Discus Dental™; Culver City, CA), and 30 similar simulated maxillary dentures were fabricated (ten of each resin denture bases that were tested). For the fabrication of the 20 dentures bases using Lucitone199® Dentsply™ (n=10) and SR Ivocap® Ivoclar-Vivadent™ (n=10), this was accomplished by pouring melted wax in the silicone mold, and fully seating a duplicate cast into the mold in alignment with the three indentations previously incorporated serving as indexes. After bench cooling at room temperature for 30 minutes, the wax-simulated dentures were removed from the silicone mold. For the fabrication of the other ten dentures using Eclipse® Dentsply™ as the denture base, a putty index of the

wax-trial complete denture was made with a high-viscosity VPS (Splash!® Discus Dental™; Culver City, CA). The index was used to fabricate the trial dentures. This system uses resin, making the melted wax technique impractical.

Figure 3. Occlusal rim.

For the fabrication of the ten complete dentures using Lucitone199® Dentsply™ as denture base, the manufacturer's recommendations were followed. Conventional detail compression molding methods and gypsum materials were used for flasking. The wax was eliminated by softening the wax in boiling water (212°F/100°C) for approximately six minutes. The flask was separated and wax was removed by flushing with a solution of boiling water to which a detergent was added. A separating agent was applied (Al-Cote® Dentsply™; York, PA) to areas of the warm mold (120°F/49°C) that contacted the resin.

Separator excess was removed with a dry brush and was thoroughly dried for (approximately three minutes following application). For the acrylic resin mixing step one unit of powder 21 grams (32cc) to 10 milliliters liquid was added and stirred sufficiently for 15 seconds to assure wetting of all powder particles. The mixing jar was covered and the material was allowed to reach packing consistency (approximately nine minutes at room temperature of 73° ± 2°F (23° ± 1°C). Resin was packed in warm flask (room temperature to 110°F/43°C). The resin dough was removed from the jar and condensed with finger pressure into the mold. The flask was closed and pressed using a hydraulic press. The flask was then submerged in water at 163° ± 2°F (73°C ± 1°C) for 90 minutes, followed by 30 minutes in boiling water (212°F/100°C). A periodic check of water bath temperature with an accurate thermometer was done. The flask was left to cool at room temperature for 30 minutes. Then it was immersed in cool water 60-80°F (16-27°C) for 15 minutes before deflasking. The processed dentures were finished using acrylic burs and polished with a wet rag wheel with slurry of coarse pumice followed by tin oxide. After polishing, all dentures were individually stored in a 200 mL beaker of distilled water at 37◦C ± 1◦C for 48 ± 2 hours.

For the fabrication of the 10 complete dentures using Eclipse® (Dentsply™) as the denture base, the manufacturer's recommendations were followed. A thin layer of separating agent (Al-Cote® Dentsply™; York, PA) was applied on the dry master cast and allowed to dry completely. The master cast was heated to 125°F (51.67°C) in the Dentsply Conditioning Oven® (Dentsply™; York, PA) set at 131°F (55°C). The temperature indicator turned black when the cast was at the correct temperature. The rounded sides of the upper or lower size baseplate resin arch were placed on the ridge and

allowed to warm on the cast for 30 seconds. Carefully the labial/buccal surfaces were adapted to avoid air entrapment. The adaptation was started at the crest of the ridge followed by the buccal slope and into the vestibule last. The same procedure was followed for the palatal/lingual section with the purpose of joining the two sides at the midline without trapping air. Eclipse Air Barrier Coating® (Dentsply™; York, PA) was applied to the entire surface of the baseplate, and placed in the Eclipse Processing Unit® (Dentsply™; York, PA). Then the baseplate cure sequence was started. After the curing cycle was completed, the baseplate/cast was removed from the unit and allowed to bench cool until reaching ambient temperature. The baseplate and master cast was soaked in tap water for five minutes to facilitate baseplate removal from the master cast. The baseplate borders were smoothed and trimmed using acrylic burs. The outer maxillary ridge area was roughened with a coarse carbide bur to enhance bonding. The entire prepared surface was cleaned with a clean denture brush and tap water; then dried. The set-up resin was softened by placing the package inside the conditioning oven at 131°F (55°C) for seven minutes. The contour resin was melted in the Dentsply Melting Pot® (Dentsply™; York, PA). The set-up resin was placed onto the ridge area of the baseplate. The surface of the set-up resin was melted with a hot air gun just prior to occlusal rim shaping. The occlusal rim was fabricated using a putty index and contoured by using the Dentsply Wax Pencil Pro® (Dentsply™; York, PA). For processing, the borders were sealed using Eclipse Gel. All external resin surfaces were covered with Eclipse Air Barrier Coating® (Dentsply™; York, PA). The denture/cast was placed on the Dentsply Conditioning Oven® (Dentsply™; York, PA) set at 130°F (55°C). Eclipse® Model Release Agent (Dentsply™; York, PA) was applied to the denture flange areas. The denture/cast was

placed in the Eclipse Processing Unit® (Dentsply™; York, PA) on the center of the turntable; then the curing process was started. When the cure cycle was complete, the dentures were allowed to cool. Finishing was performed using acrylic burs and polishing with a wet rag wheel with slurry of coarse pumice followed by tin oxide. After polishing, all dentures were individually stored in a 200 mL beaker of distilled water at 37◦C ± 1◦C for 48 ± 2 hours.

For the fabrication of the ten complete dentures using SR Ivocap® (Ivoclar-Vivadent™; NY, USA) as denture base, the manufacturer's recommendations were followed. The investment aid was placed in the lower flask half, the stone was mixed thoroughly, and the water-soaked cast was invested. Excess stone was removed from the thermal insulating component and the edges of the flask. The investment aid was removed after the stone set and the injection funnel system was positioned in its place. One injection channel was used for fanning out from the funnel tip towards the palatal portion. Prior to investing the antagonist part, the injection channels were formed with pink wax. Injection channels had a diameter of 4 mm. The upper flask half was placed on the lower half after isolating it with Ivoclar-Vivadent Separating Fluid® (Ivoclar-Vivadent™; NY, USA). The plaster was mixed and poured over the occlusal rim, avoiding the formation of bubbles. A moistened paper insert was applied as a separation layer, the flask was filled to the brim, and the cover was positioned and pressed down by hand. For the boil-out procedure, the flask was preheated in boiling water (212°F/100°C) for six minutes. The wax was removed and thoroughly boiled out with hot and clean water. Flask halves were allowed to cool to room temperature. Plaster residues were removed from the flask edges and the thermal insulation component. The still moist

plaster surfaces were isolated twice with Ivoclar-Vivadent Separating Fluid® (Ivoclar-Vivadent™; NY, USA). The capsule, composed of 20 grams polymer and 30 milliliters monomer, were mixed five minutes in the cap vibrator. The capsule was placed on the capsule plunger and the contents were pressed upward with light rocking movements. Air was allowed to escape through the capsule opening. The injection funnel was inserted in the lower flask half. The two flask halves were brought together and fitted with the flask lids. The flask was completely inserted into the clamping frame and 6000 lbs of pressure was applied with a hydraulic press (corresponds to about 80 bar / 1133 psi hydraulic pressure). At the same time, the ratchet was pushed on the clamping frame to the right. The flask was then removed from the press and the SR Ivocap® capsule was inserted in the flask. The flask was then mounted on the pressure apparatus, and this apparatus was connected with the compressed air locking valve closed to the compressed air supply (6 bar / 85 psi). The locking valve was opened. The plunger was descended and the SR Ivocap® material was pressed into the mould. The SR Ivocap® assembly was placed in a polymerization bath of boiling water (212°F/100°C) during the entire polymerization period of 35 minutes. After completion of the polymerization procedure, the SR Ivocap® assembly was removed from the boiling water and immediately cooled in cold water for 30 minutes. For deflasking, the clamping frame was mounted in the press and 6000 lbs pressure was reapplied. Pressure was released and the flask was removed from the clamping frame. The flask was then opened and the prosthesis retrieved. The processed dentures were finished using acrylic burs and polished with wet rag wheel with slurry of coarse pumice followed by tin oxide. After polishing, all dentures were individually stored in a 200 mL beaker of distilled water at 37◦C ± 1◦C for 48 ± 2 hours.

Each processed maxillary complete denture (n=30) was measured ten times by a single calibrated operator and the mean was calculated for each denture. The mean measurement of each complete denture served as the control for that specific specimen. All specimens were then subjected to microwave irradiation at 1300 W using a 1.2 cu. ft. Countertop Microwave (Panasonic™; Osaka Prefecture, Japan, *figure 4*) for three minutes immersed in sterile water based on previous protocols by Ribeiro et al[34] and Mima et al[32]. Daily microwave irradiation was simulated by allowing sterile water to reach room temperature before repeating the irradiation process. Three measurements were made of each irradiated denture at one, two, and three months. The initial measurements of each specimen (control) were compared to the measurements made at one, two, and three months.

Figure 4. Panasonic 1.2 cu. ft. Countertop Microwave (Panasonic™; Osaka Prefecture, Japan).

The protocol used for the measurements are in accordance with a previous study by Seo et al[30]. The center of the round markings were the reference point for measurements. Ten measurements were made across each dimension directly from the references points AB, AB', BC, B'C', AC, AC', and CC' with a low-angle illuminating traveling light microscope at x10 magnification (Mitutoyo Toolmakers Microscope, MITUTOYO America Corporation™) in the accuracy of 0.0005 mm *(figure 4)*. From these measurements, the mean linear dimensional change (mm) for each distance was determined and used to calculate the baseline area ABCC'B'A of the control group. Verification of the accuracy and repeatability of the measurements were accomplished by performing ten repeated measurements among the reference points. These measurements were made by a single calibrated operator so that the coefficient of variation of the repeated measures did not exceed 0.04%.

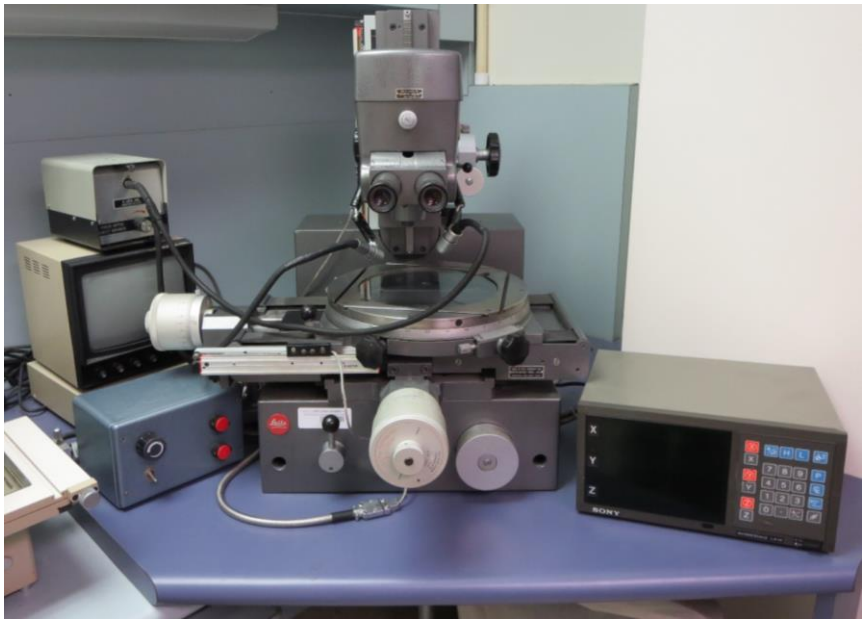


Figure 5. Mitutoyo Toolmakers Microscope (MITUTOYO America Corporation™).

For all denture bases, three measurements of each distance were made after they were submitted to the experimental condition. Measurements were made in a manner similar to those made on the control group. The round shape, which was captured by the specimens during processing, facilitated the direct comparison of the linear dimension change in each specimen. Baseline area ABB'CC'A was calculated as the sum of the area of 3 triangles (ΔABC, ΔAB'C', and ΔACC') of the control group. The area of each triangle (Δ), which had unequal sides, was estimated using the equation: $\Delta = \sqrt{S(S-X)(S-Y)(S-Z)}$ where $S=(X+Y+Z)/2$ and X, Y, and Z are the lengths of the sides of the triangle *(figure 5)*.

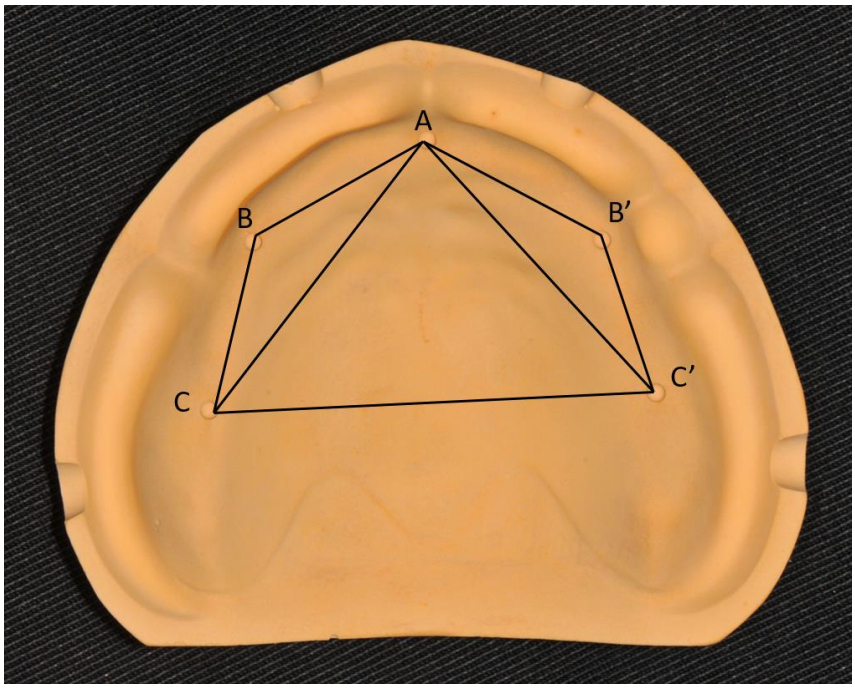


Figure 6. Triangles formed on study cast where areas were calculated.

Statistical Analysis

The overall dimensional changes of the simulated complete dentures being tested were estimated by using the percentage difference between the area of each denture base and the area of the control group. Data was statistically analyzed (SAS Version 9.2.3; SAS Institute; Cary, North Carolina) using a 2-way repeated measures ANOVA with the two factors being tested: (1) one between factor (groups) and (2) one within factor (time); followed by the Tukey HSD test to determine the significant differences between the means. An appropriate post-hoc comparison procedure was performed. Statistical analysis was conducted at the 95% level of confidence (α=.05).

CHAPTER FOUR

RESULTS

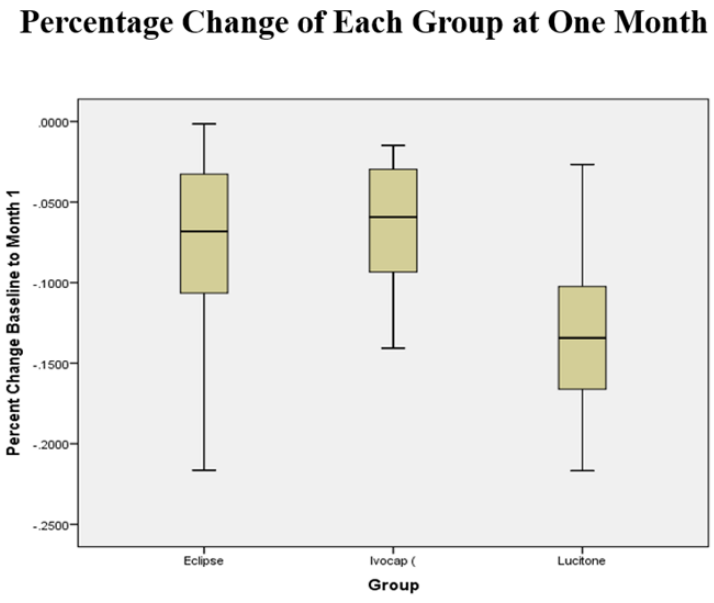
Ten dentures in each group were evaluated for percent shrinkage at one month, two months, and three months microwaving, while using the initial processing as the baseline. When comparing to the baseline, the Eclipse group had a mean shrinkage of 0.083% at one month with a standard error of 0.021; 0.14% (0.025) of shrinkage at two months; and 0.23% (0.026) of shrinkage at three months. The Ivocap group had 0.069% (0.013) shrinkage at one month; 0.16% (0.015) shrinkage at two months; and 0.25% (0.025) of shrinkage at three months. And the Lucitone group had 0.13% (0.017) of shrinkage at one month; 0.19% (0.023) of shrinkage at two months; and 0.33% (0.023) of shrinkage at three months.

Table 2. Statistical comparison of denture bases.

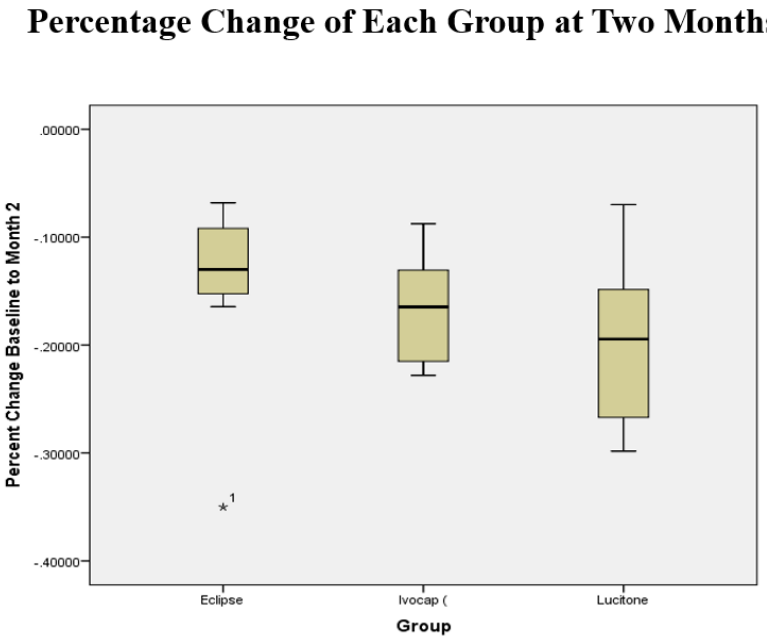
Group (Month)	Mean	Standard Error	Kolmogorov-Smirnov (P-Value)
Eclipse (1 Month)	0.083%	0.021	0.198
Eclipse (2 Months)	0.14%	0.025	0.19
Eclipse (3 Months)	0.23%	0.026	0.002
Ivocap (1 Month)	0.069%	0.013	0.200
Ivocap (2 Months)	0.16%	0.015	0.200
Ivocap (3 Months)	0.25%	0.025	0.200
Lucitone 199 (1 Month)	0.13%	0.017	0.200
Lucitone 199 (2 Months)	0.19%	0.023	0.200
Lucitone 199 (3 Months)	0.33%	0.023	0.028

A One Sample Kolmogrov-Smirnov test was performed on each group at each time point to evaluate if the data sets were normally distributed (Table 2). Comparing baseline to one month for the Eclipse group it was found that with a significance of 0.198 that the data was normally distributed. However values of 0.019 and 0.002 at two months and three months, respectively, were observed which suggests that the data was not normally distributed. A significance of 0.200 was found for all time points of the Ivocap group, suggesting that the data was normally distributed at one month, two months, and also at three months. The Lucitone group was normally distributed with a significance of 0.200 at one month and two months, but not normally distributed at three months with a significance of 0.028 (Graph 1,2,3).

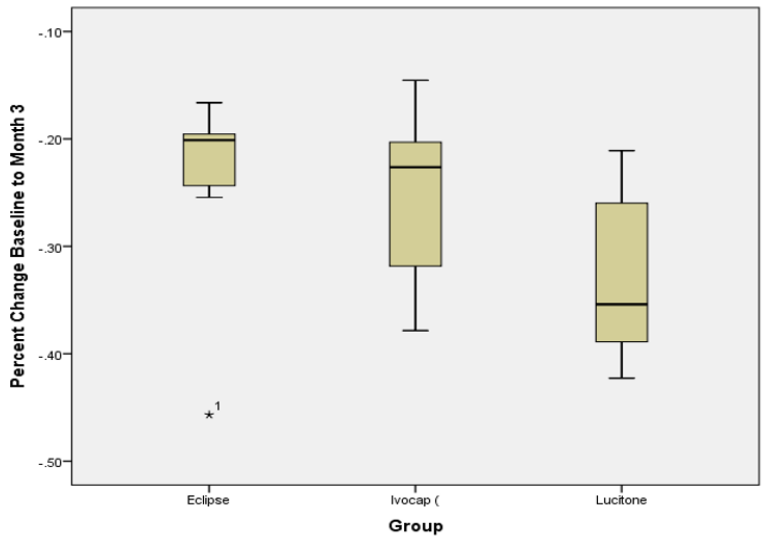
Graph 1. Percentage change of each group at one month.



Graph 2. Percentage change of each group at two months.



Graph 3. Percentage change of each group at three months.



A Related Samples Friedman's Two-Way ANOVA was used to determine if each group was statistically significantly different at each month throughout the study. When comparing the Eclipse group at baseline, one month, two months and three months a p-value of 0.000 was observed. The same p-value of 0.000 was found for both the Ivocap and Lucitone groups (Table 3,4,5).

Table 3. Eclipse group Two-Way ANOVA hypothesis summary.

Eclipse Group (Two-Way ANOVA)

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distributions of Baseline, Month1, Month2 and Month3 are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Table 4. Ivocap group Two-Way ANOVA hypothesis summary.

Ivocap Group (Two-Way ANOVA)

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distributions of Baseline, Month1, Month2 and Month3 are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.000	Accept the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Table 5. Lucitone 199 group Two-Way ANOVA hypothesis summary.

Lucitone 199 Group (Two-Way ANOVA)

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distributions of Baseline, Month1, Month2 and Month3 are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

In the present study the null hypothesis was rejected (two of three groups tested had significant differences in dimensional stability). None of the groups exceeded the average 0.5% linear polymerization shrinkage.

CHAPTER FIVE

DISCUSSION

Microwave irradiation is a proven way of disinfecting dentures. In the present study, three different types of dentures bases were evaluated for dimensional stability. After three months of daily microwave irradiation exposure, shrinkage was observed in all three groups. It was determined that there were statistically significance differences affecting the denture dimensional stability on the Eclipse and Lucitone 199 groups, and no statistically significance affecting the Ivocap group.

Although there were statistically significant differences on the changes obtained for two groups, it was determined that these changes were not clinically significant when compared to denture processing shrinkage. The initial polymethylmethacrylate formation has 21% volumetric shrinkage and 7% linear shrinkage. In order to increase the accuracy of denture bases the polymethylmethacrylate is prepolymerized to reduce shrinkage. So for a 3:1 polymer to monomer ratio the volumetric shrinkage is 6% and the linear shrinkage is 0.5%[39]. The test groups Eclipse, Ivocap and Lucitone had a total shrinkage of 0.23%, 0.25% and 0.33% respectively; each of the tested groups had less than the 0.5% linear shrinkage obtained from processing with the conventional packing technique.

The present study is in accordance with the findings of Burns et al[19] who tested three acrylic resin materials and found that they maintained excellent stability; the materials that were tested had shrinkage values in the range of 0.02% to 0.03%. They concluded that the shrinkage obtained from microwave irradiation is clinically insignificant compared to polymerization shrinkage[39]. In their study they included one

brand of the denture bases of the present study, Lucitone199® Dentsply (York, PA), but the samples used were not conventional dentures, they were cylinders uniform in size and volume, dimensions for the cylinders was 36 mm in length and 6 mm in diameter. Basso et al[36] also reported on dimensional stability, however because the shrinkage was less than 1% they determined they were not clinically significant[40].

This study also agrees with the results reported by Polyzois et al[21]. They concluded that all specimens exhibited linear changes during disinfection procedures. Although these changes were statistically significant, they were not of clinical importance (< 0.03%)[39]. However, they didn't use simulated dentures and the materials used were different from the present study. The samples used were rectangular in shape with dimensions of 65 mm of width, 10 mm of height and 2.5 mm of depth and fabricated using a heat-polymerized acrylic resin (Paladon 65, Kulzer, GmbH, Friedrichdorf, Germany).

From the results obtained in the present study, it was determined that microwave irradiation of dentures immersed in water is another technique that can be used to disinfect dentures daily over the three month test period used in this study (1300 Watts for 3 minutes). Microwave appliances are available in most homes and allows patients to have an alternative method for maintaining their prostheses.

Conclusion

Within the limitations of this study, we can conclude that:

1. Microwave irradiation revealed statistical difference on the dimensional stability of Eclipse and Lucitone 199 denture bases.

2. Eclipse demonstrated the least amount of distortion among the denture bases tested.
3. Microwave irradiation did not seem to reveal any clinical significance on the dimensional stability of Eclipse, Ivocap and Lucitone 199 denture bases when used at the wattage and time settings used in this study.

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